DRUG RESISTANCE PROFILE AMONG POST CAT II SPUTUM POSITIVE PATIENTS – CRITICAL ANALYSIS

M.D. BRANCH – XVII

TUBERCULOSIS AND RESPIRATORY DISEASES

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CERTIFICATE

This is to certify that the Dissertation "**DRUG RESISTANCE PROFILE AMONG POST CAT II SPUTUM POSITIVE PATIENTS – CRITICAL ANALYSIS"** *is the bonafide original work of* ***Dr. U. Mohamed Shafiq*** *in partial fulfillment for* ***M.D. Branch XVII (Tuberculosis & Respiratory Diseases)*** *examination of the Tamil Nadu Dr. M.G.R. Medical University to be held in March 2007. The period study was from May 2004 to March 2007.*

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Declaration

I, ***Dr. U. Mohamed Shafiq*** *declare that dissertation titled* **DRUG RESISTANCE PROFILE AMONG POST CAT II SPUTUM POSITIVE PATIENTS – CRITICAL ANALYSIS** *is a bonafide work done by me at Institute of Thoracic Medicine, Chetput and Department of Thoracic Medicine, Govt. General Hospital, Chennai under the guidance of my* ***Prof.Dr.R.ATHARUNNISA BEGUM,*** **MD, DTCD.** *The dissertation is submitted to the Tamil Nadu Dr.M.G.R. Medical University towards partial fulfillment of requirement for the award of M.D. Degree Branch - XVII (Tuberculosis & Respiratory Diseases).*

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INTRODUCTION

INTRODUCTION

Since the first appearance of Tuberculosis in humans probably some 8000 years ago its control has continued to elude the brightest minds and to challenge both the human and economic resources of the countries around the world. Several authors have estimated the magnitude of the problems in the last two decades. All these publications represent estimates but they all indicate very clearly that Tuberculosis is still one of the major killer diseases in the world wide.

It is estimated that one third of the worlds population (1.7 billion) are infected with Mycobacterium Tuberculosis. 1% of the worlds population are infected every year among these 95% of the affected individuals are in developing countries and 98% deaths due to Tuberculosis are also found to be in developing countries. These deaths accounts for 25% of all avoidable deaths in developing countries. Among the affected people 75 % are in the economically productive age.

India has 28.4 % of entire world's Tuberculosis burden. Every second one Indian above the age of 20 years is infected. There are about 14 million cases among which 3.5 million are sputum positive. Every year 2.2 Million people contact Tuberculosis. One Indian dies every minute which accounts for 5 lakhs per year.

Estimates of prevalence of drug resistance in the community will be useful for formulating policies of the treatment with highly effective regiments to ensure cure and good drug compliance by patients and prevent emergence of drug resistance.

Drug resistance Tuberculosis adds to the burden of illness in the community with the several constraints in the managements of patients. Highly efficacious treatment regimens utilizing drugs that have not been prescribed previously and known to possess good anti-mycobacterial activity need to be employed which could increase the operational costs in terms of drugs monitoring of toxicity to drugs and supervision of administration of drugs to ensure drug regularity.

Though the efficacy of chemotherapy in the treatment of pulmonary tuberculosis is well established, its application on a mass scale under the domicillary treatment programme leaves much to be desired because of the operational difficulties. With the consequent irregularity of treatment one would expect a substantial increase of excretors of drug resistant bacilli in the community. The level of primary drug resistance provides an epidemiological parameter to assess the amount of drug resistant bacillary transmission in the community.

Mycobacterium tuberculosis can develop resistance to all known anti tuberculosis drugs. Emergence of drug resistance to most potent drugs like Streptomycin and Isoniazid could be avoided by the judicious combinations of drugs with proven efficacy. Wide spread practice of prescribing monotherapy, inadequate chemotherapy, non implementation of highly efficacious treatment regimens and poor drug compliance by patients could give rise an increase in the number of patients with drug resistant Tuberculosis. This is a matter of GLOBAL concern, and more so for developing countries with limited resources. Further more a high prevalence of drug resistance could have a significant impact on the epidemiological profile of disease with an increase in the proportion of drug resistance Tuberculosis in the infector pool.

AIM OF THE STUDY

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Evaluating drug resistance profile among patients reporting at institute of Thoracic Medicine Chennai with sputum positivity after taking cat II under RNTCP and either failed/relapsed or defaulted from category II.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

DRUG RESISTANCE

By sensitive strains one means those strains of Tubercle bacilli which normally respond to low concentration of the drugs in a uniform manner. In contrast resistant strains are those which can grow in higher concentrations of the drug and they are therefore definitely different from sensitive strains. According to Dr. D.A. Mitchison 1961 resistance can be defined as a decrease of sensitivity to the drug of sufficient degree to be reasonably certain that the strain obtained is different from a sample of wild strains of tubercle bacilli of human type that have never come in contact with the drug.

The proportions of these drug resistant mutants are very small in wild strains that are not exposed to drugs. The definition of drug resistance of Mycobacterium tuberculosis was adopted by the international group of specialists assembled by the World Health Organization (WHO) in 1969. This definition was established by testing a large number of wild strains against three drugs available at that time and minimal inhibitory concentrations (MIC) of these drugs where established in Starch Free Lowenstein Jensen (LJ) medium. It was suggested that a strain would be considered resistant if one percent or more of the bacterial population was resistant to a designated concentration of drug.

With the introduction of Streptomycin for the treatment of tuberculosis Youmans and co-workers found that there was a striking clinical improvement along with a rapid decrease in the number of bacilli in the sputum. However the number of bacilli soon started increasing and the condition of the patient

deteriorated. These investigators found that bacilli isolated from the patients were resistant to higher concentration of the drug.

Pyle showed that during treatment with streptomycin alone the proportion of drug resistant bacilli increased progressively from about 1 in 88,750 organisms before therapy to about 1 in 367 after 15 weeks of treatment. Crofton and Mitchison showed that with monotherapy or inadequate therapy the number of sensitive bacilli decreased while the resistant bacilli increased in lung cavities of the patient. This was called the "Fall and Rise" phenomenon.

G. Canneti stated in 1965, bacterial resistance is as old as antituberculosis chemotherapy approximately 20 years. A glance chronologically shows that the bulk of our present knowledge atleast in its basic aspects was gained within the first ten years and that relatively little has been added since that.

While there was some disagreement about the value of susceptibility testing of the initial isolates there was no doubt about the importance of testing the isolates obtained during the course of chemotherapy.

1. If a patient does not respond clinically to treatment within a few months.

2. If sputum did not convert to smear negative within 2 – 3 months of treatment.

3. If the culture did not convert to negative within 4 – 6 months.

4. If there is an increase in the number of bacilli in the sputum after an initial decrease.

5. In case of clinical relapse.

TERMINOLOGY OF DRUG RESISTANCE

Drug Resistance in Tuberculosis could be classified as

1. Primary.
2. Acquired
3. Initial.
4. Natural

A wild strain is defined as a strain of Mycobacterium tuberculosis which has never been exposed to any anti mycobacterial drug.

1. **Primary Resistance**

Primary resistance is that which has not resulted from the treatment of the patient with the drug concerned. It includes resistance in wild strains which have never come in to contact with the drug and the resistance occurring as a result of exposure of a strain to the drug but in another patient.

2. **Acquired Resistance**

Acquired resistance is that which results from exposure of the strain to the drug and consequent selecting out of resistant mutant bacilli.

3. Initial Resistance

Initial resistance is the resistance in patients who give a history of never having received chemotherapy in the past it includes primary resistance and resistance to previous treatment concealed by the patient or of which he was unaware.

4. Natural Resistance

Natural resistance is that resistance in wild strain which have never come in contact with the drug such as Mycobacterium bovis resistant to Pyrazinamide, Mycobacterium tuberculosis resistant to Penicillin, Mycobacterium africanum resistant to Thiacetazone. Wild type resistance is the result of random mutation in naturally susceptible strain before any exposure to antituberculosis drugs.

MECHANISM OF DRUG RESISTANCE

The drug resistance in mycobacterium tuberculosis occurs by random spontaneous mutations of bacterial chromosomes which occur at a constant but very low frequency. This varies for different antituberculosis drugs. The probability of mutation of drug resistance is directly proportional to the size of the bacterial population. The development of resistance to isoniazid and streptomycin in mycobacterium tuberculosis occurs as a single step mutation.

David et-al, calculated mutation rates for many drugs using fluctuation analysis. The mutation rates are very low for most of the drugs and the mutants resistant to one of the several antituberculosis drugs appear once in every 10^7 cells. Thus emergence of the drug resistance is due to the selection of pre-existing resistance mutants in the original bacterial population. In clinical practice combinations of two or more antituberculosis drugs are given to the patients in order to eliminate all mutants resistant to any of the drugs.

The rates of spontaneous resistance are 1 in 10^6 organisms for Isoniazid, 1 in 10^8 for Rifampicin, and 1 in 10^6 for Ethambutol and 1 in 10^5 for streptomycin. Assuming they are independent events the probability of resistance to more than one drug is the probabilities for each drug alone. The probabilities of isoniazid and rifampicin resistance occurring in the same organism is 1 in 10^6, 1 in 10^8 respectively or 1 in 10^{14}.

The bacterial population in a cavitary pulmonary lesion is estimated to be 109 organisms. Therefore the bacterial population of these lesions is lightly to include a small number of mutants resistant to any single antituberculosis drug. Only very rarely will the population include a significant number of mutants resistant simultaneously to two or more drugs. Monotherapy with a single antituberculosis drug does not induce drug resistant mutants. But it suppresses the bacteria susceptible to that drug thereby selecting for mutants resistant to that drug.

Drug resistant tuberculosis occurs when there is a substantial increase in the proportion of the organisms that are resistant to one or more antituberculosis drugs.

There are two ways a patient can develop drug resistant tuberculosis. Acquired or secondary drug resistance occurs when the small numbers of drug resistant mutants are selected as a result of ineffective anti tuberculosis. In tuberculosis acquired drug resistance may appear after two weeks but more usually from one to four months after the start of therapy when the bacterial population is relatively large.

Primary drug resistance on the other hand occurs when the patient becomes infected with mycobacterium tuberculosis organism resistant to one or more drugs before the patient is treated with the drug in question. Primary drug resistance is not distinguishable clinically from acquired drug resistance except by history.

Initial drug resistance is an entity which includes all primary drug resistance and also concealed acquired resistance. This sort of concealed acquired resistance will pose a major threat to the outcome of treatment. For example, the risk of relapse with isoniazid resistant organism increases by about 4 % per month or prior therapy, but upto 23 % of patients in one study treated for one month with isoniazid alone had isoniazid resistant organism.

In clinical practice a patient with tuberculosis is said to have drug resistant disease if that patients bacillary population consist of organism that would probably failed to respond treatment with drug concerned in normal dosage, for example a dosage that will cause a response in patients infected with drug susceptible organism.

PROPOSED THEORIES

Very little is known regarding the mechanism of the development of drug resistance to various antituberculosis drugs. Of the several theories put forward to explain the mechanism of development of drug resistance in micro organisms, three theories may be relevant to mycobacteria. These include

1. Interference in uptake or penetration of the drug in to the bacterial cell.
2. Development of insusceptible metabolic pathways.
3. Destruction of drug.

Using the ^{14}C labeled isoniazid Barclay et-al found that isoniazid resistant strains take up markedly less radioactive material than the susceptible strains. Youatt et-al confirmed these findings and suggested that the possible reason could be due to alteration in cell permeability.

Factors Influencing the Development of Acquired Drug Resistance

INCREASED CHANCE

1. Previous treatment of Tuberculosis.
    - 1.1 Greatest risk if treatment fails or disease relapses while patient is still on drugs.
    - 1.2 Significant risk if treatment was inadequate. This includes weak drug regiments and / or inappropriate duration.
    - 1.3 Treatment received while living in high tuberculosis incidence area.

2. Birth and / or recent residence in a high tuberculosis incidence area.

   2.1 Particularly Asia, South and Central America and Africa.

   2.2 Certain localized areas within developed countries like Moscow, Latvia, Eistonia, Dominican republic, Ivory coast. These are called as hot zone for MDR TB by WHO. In India Places like Mizoram, Assam.

   2.3 The younger the age the greater the risk.

3. Recent exposure to a known case of drug resistant disease.
4. Infection with mycobacteria other than mycobaterial tuberculosis.

DECREASED CHANCE

1. No previous treatment for tuberculosis.
2. Birth and / or resistance in low tuberculosis incidence area -- the longer the less likely.
3. Recent exposure to a known case of drug susceptible disease.

MULTI DRUG RESISTANT TUBERCULOSIS (MDR TB)

MDR TB is defined as mycobacterium tuberculosis resistant atleast to isoniazid and rifampicin with or without resistance to other drugs.

IMPLICATIONS OF DRUG RESISTANCE

1. The most important implication of drug resistance (primary and / or acquired including MDR) is the outcome of the treatment. The

response in patients harboring multi drug resistant organism is very poor both in immuno competent and immuno compromised patients.

2. The second and equally important implication is the spread and transmission of drug resistant tuberculosis. This is much more dangerous than the previous implication. The seriousness of the spread of drug resistant tubercle bacilli is magnified several fold when patients involved are infected with HIV.

GLOBAL PREVALENCE OF DRUG RESISTANT TUBERCULOSIS

1. From 1982 to 1986 the CDC evaluated drug susceptibility of mycobacterium tuberculosis isolated from each 3730 new never treated patients. The overall resistance to atleast one drug was 8.8 % with isoniazid (5.3 %) and streptomycin (4.9 %) being the most common.

2. CDC found that primary drug resistance varied by ethnicity with rates for Asians 14.8% and Hispanics 11.8% being lower for the whites 4.9% than blacks 6.1%.

PREVALANCE OF DRUG RESISTANCE IN INDIA

1. In the1960s Indian Council of Medical Research (ICMR) conducted two nation wide surveys at 9 urban chest clinics in India. The results of the first survey showed the resistance level of 8.2 % to isoniazid alone 5.8 % to streptomycin alone and 6.5 % to both the drugs.

2. A decade later a study was conducted to assess the prevalence of primary drug resistance in Government chest Institute and chest clinic

of Government Stanley Hospital Chennai. The results of the study were almost similar to the earlier ICMR surveys and authors reported that prevalence of primary drug resistance has not risen during the span of ten years.

3. In the early 1990s a retrospective study done at New Delhi showed a high level of primary drug resistance to isoniazid (18.5%) at a low level of rifampicin.

4. Overall the prevalence rate of primary drug resistance to isoniazid as a single agent ranged from 6 to 13%, streptomycin as a single agent ranged from 1 to 5.8 % and rifampicin from 0 to 1.9 %.

TRC STUDIES ON PREVELANCE OF PRIMARY DRUG RESISTANCE

It clearly shows that there was a gradual increase in the prevalence of primary drug resistance to anti tuberculosis drugs. For isoniazid the resistance rates ranged from 3 to 17 %, streptomycin from 3 to 13 % and rifampicin remains at 1 %.

ACQUIRED DRUG RESITANCE

A study was conducted by the ICMR to compare the efficacy of short course chemotherapy with the conventional non short course chemotherapy in North Arcot District in Tamil Nadu. It was found that there was an increase in frequency of acquired drug resistance with 67% resistant to isoniazid 26 % to streptomycin and 12% to rifampicin, 6% resistant to both isoniazid and rifampicin. A study conducted by the Institute of Thoracic Medicine Chennai showed that acquired resistance was 63% out of which 23.5 % were resistant to single drug and 39.5 % resistant to more than 1 drug.

The overall rates of acquired resistance to isoniazid ranged from 34.5 to 67% for streptomycin from 26 to 26.9 % and for rifampicin from 2.8 to 37.3 %.

INITIAL DRUG RESISTANCE

Overall Initial resistance to isoniazid as a single agent ranges from 0.62 to 13.2 % to streptomycin from 2.2 to 7 % and to rifampicin from 0 to 1.7 %.

MULTI DRUG RESISTANCE

The rate of MDR TB in India is very low ranged from 0 to 6 %. Primary MDR TB was found to be less than or equal to 3.2 %. Acquired MDR TB is less than or equal to 6 % except in Gujarat where it is 18.5 %.

In a recent report from Institute of Thoracic Medicine Chennai on the prevalence of MDR TB among patients undergoing treatment for varying periods of time at 4 district Tuberculosis centers in Tamil Nadu showed that 20.3 % were found to be harboring. Majority of these patients are irregular and interrupted treatment owing to non availability of drugs. Overall MDR TB is around 13.3 % of all TB patients.

EXTENSIVELY DRUG RESISTANT TUBERCULOSIS

A serious wake-up call for global health, **T**uberculosis outbreaks in the developed world are newsworthy. However, in the developing world, where deaths from tuberculosis are common, it takes something exceptional for an outbreak to

attract much attention. In response to a recent report at the 16th international AIDS conference and to increasing South African media reports, the World Health Organization expressed concern about extensively drug resistant tuberculosis (also referred to as "XDR tuberculosis").

Among 536 culture confirmed cases of tuberculosis at a rural hospital in South Africa, **41% were multidrug resistant**, defined as resistance to rifampicin and isoniazid (two key first line drugs). This is cause enough for concern as multidrug resistant tuberculosis has a worse outcome and its management is very difficult even in high resource settings. Even more alarming was that 53 (24%) of the isolates from multidrug resistant tuberculosis fulfilled the **definition of extensively drug resistant tuberculosis namely, multidrug resistant tuberculosis that is also resistant to at least three of the six classes of second line agents**. Such tuberculosis is virtually untreatable.

All patients in this outbreak who were tested were HIV infected, and 52 of the 53 died after a median of just 25 days. In 90% of the isolates the same genetic fingerprint was present, indicating extensive recent transmission. Fifty six per cent of patients had previously been admitted to hospital, raising the likelihood of nosocomial transmission.

Outbreaks of infectious diseases are always more newsworthy if their implications extend beyond the local context, which is the case with extensively drug

resistant tuberculosis. For some years, such strains have been known to exist in Asia, North and South America, and Europe. In March this year, the Centers for Disease Control and Prevention and WHO reported a survey of over 17 000 tuberculosis isolates collected from around the world between 2000 and 2004. Overall, 2% of multidrug resistant strains were also extensively drug resistant, being most frequently found in eastern Europe, western Asia, and South Korea. Population based data from the United States, Latvia, and South Korea showed that 4%, 19%, and 15% respectively of multidrug resistant strains could be defined as extensively drug resistant.

The epidemiology and the limited genotypic data currently available indicate that this is not a single strain, but that extensively drug resistant strains are likely to have emerged in many different places and on multiple occasions. Paradoxically, this is both reassuring and alarming. It is reassuring in that the emergence of extensively drug resistant tuberculosis in more than one strain suggests that the mutations responsible are specific for drug resistance rather than reflecting a fundamental change in behaviour of the organism. This is nevertheless alarming because it also suggests that extensively drug resistant tuberculosis probably arises fairly regularly and is already disseminated.

Drug resistance to tuberculosis results largely from poorly managed care and control of the disease. Poor prescribing practices, low drug quality (or erratic supply), and suboptimal adherence can all contribute to this. Bacilli are subject to intense drug

selection, and exposure to mono-therapy predisposes to an accumulation of mutations that confer resistance. **Hence optimal treatment includes four drugs to which the organism is sensitive, and a single drug should never be added to a failing regimen. In much of the world, routine culture and sensitivity testing is not available. Thus, where multidrug resistant tuberculosis emerges, inappropriate treatment regimens may lead to serial acquisition of resistance mutations, with potential for emergence of extensively drug resistant tuberculosis**. Widespread use of second line tuberculosis drugs (such as quinolones for respiratory tract infections) may also contribute to the development of resistance. Thus, the emergence of extensively drug resistant tuberculosis should come as no surprise it was entirely predictable in the context of poor control practices.

The havoc that institutional transmission of multidrug resistant tuberculosis can wreak amongst HIV infected people was evident in the US in the early 1990s. The very modest actual rise in the incidence of tuberculosis that coincided with these outbreaks has now been reversed, albeit with extraordinary effort and cost. However, the huge potential for extensively drug resistant tuberculosis to further undermine control practices in communities in South Africa and elsewhere in the region is self evident and would be much more difficult to control. In some communities with an antenatal prevalence of HIV of 30%, annual notification rates for tuberculosis have already increased uncontrollably over the past 10 years, reaching 1500/100 000-a rate more than 250 times higher than rates in the US. Extensively drug resistant tuberculosis must now serve as a serious wake-up call. Although the potential consequences may be most grave in settings with a high prevalence of tuberculosis

and HIV, extensively drug resistant tuberculosis is nevertheless already a very serious development in many other parts of the world too. What response is needed? The global scale and molecular epidemiology of extensively drug resistant tuberculosis require urgent assessment, and laboratory capacity needs to be greatly increased within a network of sentinel sites. Control practices must be rigorously and effectively implemented. Increasing cure rates for tuberculosis through directly observed treatment short course (DOTS) is crucial. Detection rates for cases of tuberculosis need to be improved, highlighting the need for a new diagnostic test. Technologies that can determine the presence of drug resistance at the point of care are needed, as are new drug treatments. The DOTS-Plus strategy for treatment of multidrug resistant tuberculosis needs to be further developed for areas where the disease is established. Nosocomial transmission of tuberculosis is probably commonplace in the developing world, and simple, effective strategies to reduce such transmission need to be urgently implemented. More fundamentally, the emergence of extensively drug resistant tuberculosis is a reminder that tuberculosis needs massive broader commitment: the incompletely funded Global Plan to Stop TB demands political will and financial action.

MATERIALS AND METHODS

MATERIALS AND METHODS

1. The study was conducted at Institute of Thoracic Medicine Chennai.

2. Patients who had reported with sputum positivity with the H/O treatment under RNTCP with cat II and subsequently failed/defaulted/relapsed were included in the study.

3. All of them were subjected to sputum AFB culture and sensitivity studies.

4. Sensitivity pattern were evaluated with their previous ATT prior to cat II. Total of 140 patients were included, 34 were excluded based on the exclusion criteria. The study conducted during 2004 & 2005. The remaining 106 patients were evaluated in this study.

5. It is a retrospective study cohort. The culture sensitivity obtained were analyzed retrospectively.

INCLUSION CRITERIA

Patients who had reported with sputum positivity with history of treatment under RNTCP with Cat II and subsequently failed / defaulted / relapsed were included in the study.

EXCLUSION CRITERIA

The following were the exclusion criteria.

1. Those who had diabetes mellitus (18 patients) at the time of enrollment into the study.

2. Those who had HIV positivity (6 patients) at the time of enrollment into the study.

3. Those patients whose AFB sputum cultures were contaminated (10 patients).

SPUTUM FOR AFB CULTURE AND SENSITIVITY

Sputum for AFB culture and sensitivity for 106 patients who were included in the study were done at the Tuberculosis Research Centre (TRC).

PROCEDURE

Cultures were put up by single step culture technique using transport medium.

PREPARATION OF TRANSPORT MEDIUM

This consist of TRISODIUM PHOSPHATE ($Na_3PO_412H_2O$) 200 gms, Ammonium sulphate – 5 gms magnesium sulphate -500 mgs, Ferric ammonium Citrate – 250 mgs and distilled water 100 ml.. These chemicals were dissolved in water by heating, the solution was filtered and sterilized at 121 degree centigrade for about 15 minutes. The medium termed transport medium was distributed in 10 ml. quantity in to sterile screw cap Mc Cartney bottles and stored at room temperature. These bottles were supplied to the patients who were instructed to open them and expectorate sputum in to them.

SINGLE STEP CULTURE TECHNIQUE

Sputum was either mixed with two volumes of transport medium or collected in to Mc Cartney bottles as described above. The sputum transport medium mixture is centrifuged and allowed to stand overnight at room temperature on the following morning the supernatant fluid was decanted and one loop full of the deposit was inoculated on to the slope of Lowenstein Jensen (LJ) medium which was then incubated at 37 degree centigrade for 6 weeks.

CULTURE READING

Culture was read at the end of every week for six weeks and growth typical of mycobacterium tuberculosis were noted. It is rough and tough white colored colonies sometimes like cauliflower appearance and it is graded as follows.

+ + +	Confluent Growth
+ +	Innumerable Discrete colonies
+	20 – 100 Colonies
Less than 20 colonies	Actual Number

Cultures that did not show any growth at the end of 8 weeks were declared as negative. All the positive cultures were tested for sensitivity to streptomycin, isoniazid, rifampicin, ethambutol, ethionamide, ofloxacin, and kanamycin.

The sensitivity pattern was done using absolute concentration method and resistance ratio method.

Absolute concentration method

Absolute concentration method test determines the minimal inhibitory concentration of isoniazid and streptomycin by the inoculation of control media and the drug containing media with a carefully controlled inoculum of mycobacterium tuberculosis. Media containing several sequential two fold dilutions of each drug are used. Resistance is indicated by the lowest concentration of drug which will inhibit growth defined as 20 colonies or more at the end of 4 weeks.

Resistance Ratio Method

Resistance Ratio Method is a variant of absolute concentration method was first introduced by Mitchison to prevent variation of MICs when a strain of mycobacterium tuberculosis was tested on different batches of medium because of fluctuation in the degree of inactivation of streptomycin by the egg components of the medium during inspissation.

Intra laboratory and inter laboratory variations between media batches with regard to the drug susceptibility testing of streptomycin and other drugs were corrected after comparing the resistance of the wild strain with reference strain $H_{37}RV$ tested in parallel on the same batch of the medium.

Growth is defined as presence of twenty or more colonies at the end of four weeks. The resistance ratio is defined as minimal concentration inhibiting the growth of the test strain divided by the minimal concentration inhibiting the growth of the standard susceptible strain in the same set of tests. A culture showing a resistance

ratio of 2 or less is defined as susceptible while a ratio of 8 or more denotes resistance.

RAPID METHODS FOR THE DETECTION OF DRUG RESISTANCE IN MYCOBACTERIUM TUBERCULOSIS

1. Radio Metric Methods – BACTEC
2. Luciferase reporter Assay
3. Mycobacteria Growth Indicator Tube
4. Rapid Genotype Based Novel Techniques - using polymerase chain reaction (PCR)
5. Restriction Fragment Length in Polymorphism Analysis (RFLP)

RESULTS

RESULTS

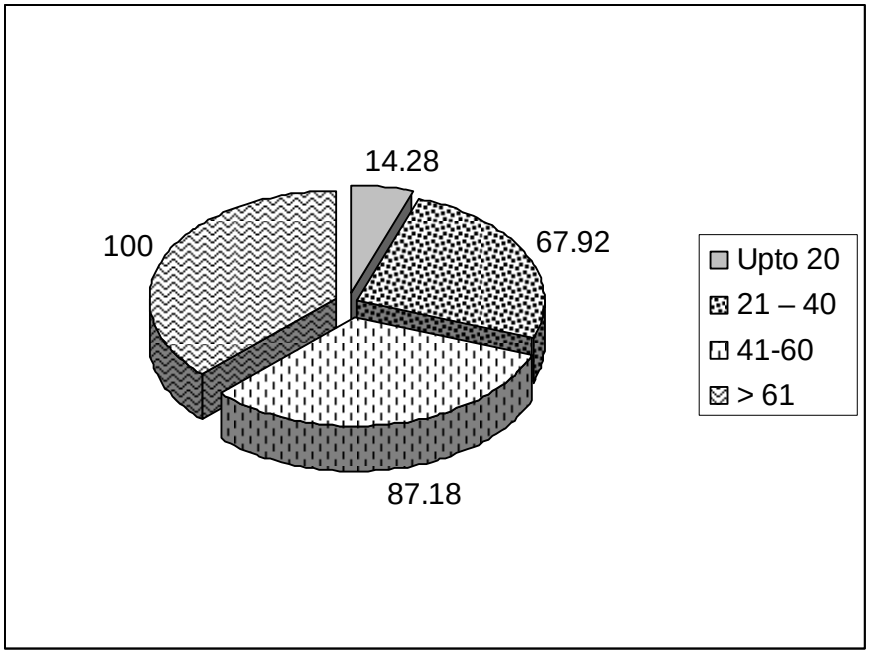
TABLE 1

AGE – SEX DISTRIBUTION

Age group years	*Male*		*Females*		*Total*	
	Patients	*%*	*Patients*	*%*	*Patients*	*%*
Upto 20	1	14.28	6	85.72	7	6.60
21 – 40	36	67.92	17	32.08	53	50.00
41-60	34	87.18	5	12.82	39	36.80
> 61	7	100	0	0	7	6.60
Total	**78**	**73.58**	**28**	**26.42**	**106**	**100.00**

1. Total number of patients studied were - 106.
2. Among them male patients were - 78.
3. Females were – 28.
4. More number of patients were in the age group of 21 to 40.
5. Total number of patients in the above group were 53.

Sex Distribution - Male



Sex Distribution - Female

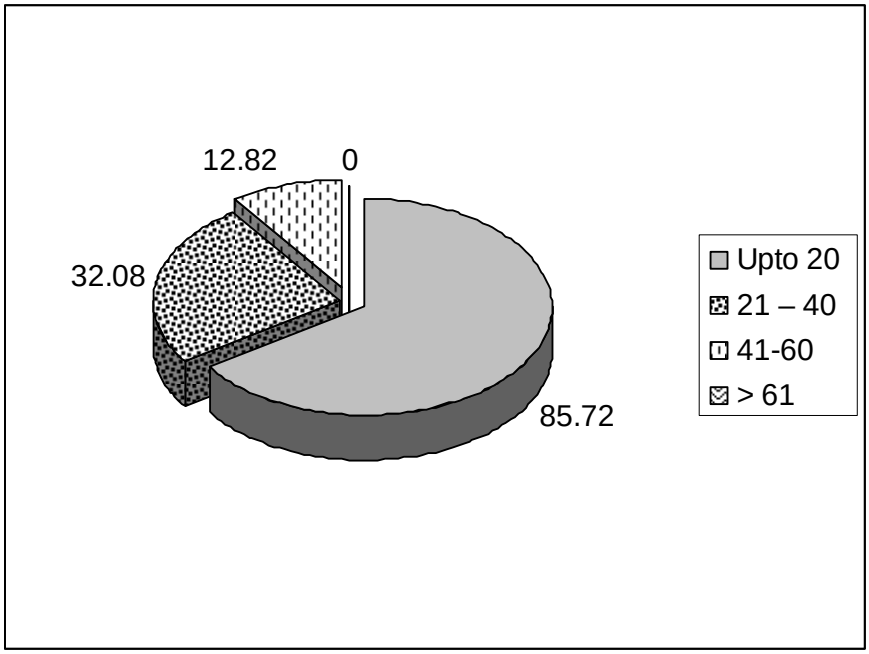


TABLE 2

Drug Resistance Profile of the study population:

Category II status	*Number*	*MDR*	*% of MDR*	*One or more drug resistance*	*Percentage*
Failure	90	78	86.66	12	13.33
Relapse after cat II	1	1	100	--	
Treatment after default from cat II	15	10	66.66	5	33.33
Total	106	89	83.96	17	16.04

1. Among the 106 patients who were included in the study 90 have failed with Cat II and subsequently 78 have become MDR TB patients which accounts for 86.66 %

2. 15 patients were in the treatment after default group after Cat II. Most of them have defaulted atleast two times after initiating Cat II. Among the 15, 10 have become multi drug resistant tuberculosis patient which accounts for 66.66 %.

3. Among the patients who failed with Cat II the remaining 12 non MDR TB patients had resistance to one or more other drugs.

4. Among the patients who were in the treatment after default group following Cat II the remaining 5 non MDR TB patients had resistance to one or more other drugs.

TABLE 3

Cat II Failure – Non MDR TB patients drugs resistance profile:

Drugs	*No. of Patients*
H	3
S, R	1
R, E, ETH, KAN	1
S, H	3
H, ETH	2
H, E, ETH	2

1. All patients had resistance to atleast one of the primary drugs.
2. Three patients had reserve drug resistance namely ETH & KAN.
3. All the patients had either INH or Rifampicin resistance.

Post cat II treatment after default non – MDR (5)

1. 4 of them had only INH resistance.
2. 1 had both INH & ETH resistance.

TABLE 4

Cat I outcome of these 106 patients.

Outcome	No. of patients	No. of MDR	Percentage
Failure	75	65	86.66
Defaulter	15	11	73.33
Cure followed by relapse	16	13	81.25
Total	106	89	

1. Among the 106 patients studied 75 patients have failed with Cat I. and subsequently 65 have become MDR TB patients which accounts for 86.66 %.

2. Among the 106 patients 15 have defaulted and 11 have subsequently have become MDR TB patients.

3. Among the 16 relapsed cases 13 have become MDR TB patients.

TABLE 5

Category II Failure & Defaulter Rates – National Figures

YEAR	2003	2004
NO OF RETREATMENT CASES	112304	150297
FAILURE %	6	6
ACTUAL NO. FAILURE	6738	9018
DEFAULTER %	15	15
ACTUAL NO. DEFAULTER	16846	22545

1. The actual number of cases registered under Cat II is showing an increasing trend.
2. The actual number of cases failed with Cat II in terms of absolute number has also increased.
3. Percentage of Defaulters in terms of absolute number has also increased.

DISCUSSION

DISCUSSION

Information on the prevalence of the drug resistance is essential for the successful formulation of treatment policies in our country.

Sputum positive re-treatment cases constitute 25% of all smear positive cases registered under RNTCP. Our country is progressing towards 100% implementation of RNTCP. In this background as each year progresses the no. of cases put on cat I as well as cat II are showing an increasing trend. Those cases who fail with cat II as well as those who default from cat II add to the newly detected cases each year.

We are in need of intermediate reference laboratories for carrying out drug sensitivity testing for tackling cat II failure and treatment after default groups in every state. Those cases thus identified require reserve line of drugs which are costlier as well as not freely available.

In this study among cat II failure the prevalence of MDR TB to the tune of 87% is a cause for concern. Hence advising culture and sensitivity at an appropriately early state i.e) at the time of cat I failure are atleast when the sputum conversion does not occur at the end of intensive phase will help to diagnose multi drug resistance or drug resistance patients under program condition. It will enable us to give the correct line of management either before or at the time of declaring a patient as cat II failure.

RELATED STUDIES

1. **INFLUENCE OF DRUG SUSCEPTIBILITY ON TREATMENT OUTCOME AND SUSCEPTIBILITY PROFILE OF 'FAILURES' TO CATEGORY II REGIMEN.**

This study was done by Tuberculosis Research Centre Chennai. From May 1999 through December 2004, a total of 697 smear-positive patients were started on re-treatment with CAT-II regimen. The proportion of smear-positive re-treatment cases to the total smear-positive cases ranged from 24.5% in 1999 to 22.8% in 2004. The 697 patients included - 32% cases of 'Relapse', 20% 'Failure' and 47% 'Treatment After Default' cases.

Treatment outcome according to 'type' of cases Of the 697 smear-positive patients registered to CAT-II regimen, 572 patients whose treatment outcome was available by December 2004 are included in the analysis. Of the total 572 cases, 238 **(42%) had a successful treatment outcome (cure-41%, treatment completed-1%) and 240 (42%) defaulted. 'Relapse' cases had a significantly higher cure rate (51%) compared to 'Failure' and TAD cases** ($p<0.01$). Fifty-two (9%) patients failed to the re-treatment regimen; 14% (15 of 111) among 'Failures', 8% (15 of 187) among 'Relapses', and 8% (22 of 274) among 'TAD' cases. However the difference was not statistically significant. The time at which patients defaulted was available for 219 out of the total 240 from the treatment cards and **49% defaulted** within the first 3 months of treatment. The rate and time of default were almost similar in all types of patients. **Forty-one (7%) patients died** during the course of the treatment. Of the 37 patients for whom information is available, **70% died within 3 months of starting treatment**.

The proportion of smear-positive retreatment cases in this DOTS implemented area, over a period of 5 years, from 1999 through 2004, did not show any significant changes and it ranged from 24.5% in 1999 to 22.9% in 2004. Among the retreatment TB patients, nearly 50% constituted patients who came for re-treatment after defaulting to the previous regimen.

The **low success rate (42%) to the CAT-11 regimen was mainly due to the high default (42%) during treatment**. If all these defaulted patients (240) also had been regular for treatment, the treatment success would have been 72%. Another important finding revealed in this analysis is that the prevalence of drug resistance (non-MDR as well as MDR) was almost similar initially and at the time of failure. Development of resistance to Rifampicin among patients who failed to CAT II regimen was low (2 patients with initial resistance to H emerged resistance to R.)

High default rate (42%) was the major reason for the low cure rate in this area. Default rate was similar in all groups of patients, irrespective of the type of patients or their drug resistance pattern. **The success rate (42%) for the re-treatment cases in this report is significantly low compared to the national average of around 70%**

2. IS IT WORTH TREATING CATEGORY I FAILURE PATIENTS WITH CATEGORY II REGIMEN?

This study was done by Tuberculosis Research Centre Chennai. In all, 1463 study patients were registered between May 1999 and December 2002. Sputum was collected from 1395 (95%) and drug susceptibility profile was

available for 1226 patients, 158 were negative on culture and 11 were contaminated. Of the 1226, 1094 (89%) had organisms susceptible to H and R, 111 (9%) resistant to H, 16 (1.3%) to H and R (Multi-Drug Resistant) and 5 (0.4%) to R alone. Treatment outcome of the 1463 patients was as follows: 1117 (76%) had a successful treatment outcome, 212 (14.5%)had defaulted, 58 (4%) died, 74(5%) were declared to have failed while two were transferred out.

Under programme conditions, patients are declared 'failed' on Cat I regimen if smears become positive at 5-month or more after starting treatment. In our series, of the 74 patients declared to have 'failed', MDR TB (organisms resistant to H and R) was seen among 17% justifying the use of Cat II regimen for failures of Cat I treatment for the remaining 83% of patients. Thus, all failures do not have MDR TB. A study from Vietnam had reported 80% of Category I failure cases to have MDR TB. The regimen, 2SHRZ/6HE used for treatment in Vietnam differed from the 2REHZ3/HR3 used in this programme. In the study from Vietnam, emergence of drug resistance was considered only for patients who had RFLP matching cultures at pre-treatment and at the time of failure. A programme-based study from Malawi had reported that none of the failures to first line treatment with 6HE in the continuation phase following an intensive phase of either 2SHRZ or 2HRZE3 had MDR TB.

In conclusion, our finding that nearly 80% of the 'failures' (as declared in the programme, based on smear results), have organisms susceptible to R, justifies the use of the currently recommended category II regimen for failures

of category I treatment. Close monitoring of these patients will be required to identify failures early and if necessary, **change of treatment can be considered for those patients who do not show any response to treatment with Cat II regimen at 3-months.**

3. **TIME TO ABANDON THE STANDARD RETREATMENT REGIMEN WITH FIRST LINE DRUGS FOR FAILURE OF STANDARD TREATMENT**

This study was conducted in Vietnam. About 2901 new cases were registered and sputum samples were collected before initiating the treatment with Cat I and at that time of failure / relapse once again sputum samples were collected. Both the samples underwent culture and sensitivity and RFLP studies.

80 % of the failure cases in Cat I were MDR TB patients. Therefore culture and sensitivity at the end of intensive phase if sputum remains positive or at failure in Cat I is recommended.

Cure rate of failure cases treated with re-treatment regimen of first line drugs in Vietnam was 46% and Peru 47%. Therefore who fail the treatment regimen with the first line drugs and receive poor re-treatment regimen based on same drugs will most likely fail.

4. **DRUG SUSCEPTIBILITY AMONG FAILURES AND RELAPSES CASES OF TUBERCLOSIS** : IS STANDARD RE-TREATMENT REGIMEN ADEQUATE

This study was conducted in Vietnam, objective of the study was to determine acquired drug resistance among failure and relapsed cases after treatment of new smear positive tuberculosis patients.

Conclusion of the study showed that primary drug resistance was a strong risk factor for failure and relapse and for acquiring further resistance. As 80 % of failure cases had MDR the standard re-treatment regimen appears inadequate for failure cases in control programs with a very high cure rate among new cases.

5. OUTCOME OF TUBERCULOSIS RETREATMENT IN ROUTINE CONDITION IN COTONOU, BENIN

The aim of the study was to evaluate the patient characteristics and outcome of Tuberculosis re-treatment regimen in a well functioning national tuberculosis program.

It is a retrospective, register based study. All smear positive pulmonary tuberculosis patients put on re-treatment regimen (CAT-II) were included. Among the 8103 patients registered 642 put on re-treatment. This study was done during 1992 – 2001.

Among the 642 patients put on re-treatment regimen 236 patients were studied. Among them 113 had relapse 84 failure 39 return after default. Most of the relapse 57 % and return after default 72 % were put on re-treatment within one year. Overall re-treatment results were satisfactory with 78 % success, 3 % failure and 21 % defaulter.

The conclusion of the study was that standard re-treatment regimen was effective in Cotonou probably because the NTP is functioning well, there are no drug shortages, drug taking is strictly supervised and good treatment plan is followed.

CONCLUSION

CONCLUSION

The drug resistance profile among patients reporting Institute of Thoracic Medicine Chennai with sputum positivity after taking CAT II regimen under RNTCP and either failed / relapsed or defaulted from CAT II were evaluated and the following conclusions were made.

1. Possibility of MDR TB is found to be 87% when a patient fails with cat II under RNTCP.

2. Possibility of having MDR TB is to the tune of 90% when a patient fails with CAT II preceded by a failure in CAT I

3. It is preferable to have culture and sensitivity study when the patient fails with cat I or atleast when the patient does not get converted at the end of intensive phase of cat II.

By doing so unnecessary time delay in initiating appropriate reserve regimen can be avoided and can prevent the diseases transmission to others.

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